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THE ROLE OF SPECIFIC FATS IN COMPLEMENT FIXATION

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In previous articles[1] it has been shown that the fats peculiar to the gonococcus served as antigen in complement fixation tests for gonorrhea to a degree better than aqueous or salt solution suspensions or autolysates of the germs. To test whether the antigen emlpoyed in the serum test for syphilis also depends for its value on a specific fat content a long series of tests was made[2] from which it was determined that such was the case, the specificity depending on a definite fatty composition or configuration of the lecithin-like substance.

Since then the work has been extended to include antigens for cholera, typhoid and tuberculosis.

For the tests with the fat of the cholera vibrios there were used an Austrian strain of virulent organisms and guinea-pig immune serum. Mass cultures on neutral nutrient agar in Roux flasks were washed off in small amounts of distilled water, completely saponified by potassium alcoholate with heat under 100 C. and the soaps recovered as fatty acids, after acidification, by solution and washing in dry ether. The ethereal solutions were then concentrated to dryness in vacuo, the fatty acids taken up in alcohol, converted into the sodium salts by sodium carbonate, the salts repeatedly recrystallized from alcohol, dried, weighed and redissolved in absolute alcohol. These salts were free from protein.

The salts were preferred to the fatty acids themselves as previous experience had shown them to yield better complement fixation. A series of fixation tests was then carried out on the immune cholera serum of guinea-pigs, the antigen consisting of the alcoholic solution of the specific cholera fats of which solution 1 c.c. contained 0.002 gm., the hemolytic system being the sheep-rabbit system, and the complement

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[1] Warden: Jour. Infect. Dis., 1915, 16, p. 426; Jour. Am. Med. Assn., 1915, 65, p. 2080; 1917, 68, p. 432. Warden and Schmidt: Jour. Lab. and Clin. Med., 1916, 1, p. 5.

[2] Warden and Kelly: Unpublished work.

fresh guinea-pig serum. All the reagents were titrated in the usual manner. The tests showed that complement was bound in the first incubation by the specific fat-sensitizer (amboceptor) complex formed, for the subsequent addition of sensitized sheep cells showed no hemolysis, or the addition of sensitized cholera vibrios gave no Pfeiffer phenomenon. The following specimen protocols illustrate these facts, but before considering the application of this principle to the organisms mentioned it is advisable at this point to regard its relation to cells other than bacteria. If it be true that definite fat complexes of bacterial cells are able to replace the cells themselves as antigen in the serum test we should expect an analogous phenomenon to obtain with respect to the red blood cells serving as antigen in the hemolytic system. That such is the case was shown by suitable experiments. When the fats specific to the red blood cells (sheep) or their stromata,[3] in the form of an alcoholic solution of the sodium salts (or with alcohol-ether extracts) were incubated with sensitizer and complement, the complement was found to be absorbed, so that on the subsequent addition of sensitized corpuscles, or of sensitized cholera vibrios, there was no hemolysis or Pfeiffer phenomenon.

CORPUSCLE ANTIGEN

Alcoholic solution of fat antigen, prepared as outlined, 1 c.c. contained 0.002 gm. Antigen neither hemolytic or anticomplementary in working doses. Dose employed, by weight, 0.0000112 gm. (sufficient to bind total complement).

Fresh guinea-pig serum, 5 standard drops of one-tenth dilution in salt solution, varying with titration, used as complement.

Antisheep-rabbit serum, 1 standard drop, 0.02 c.c. or 2 units.

Corpuscle suspension—2% suspension in salt solution of fresh washed sheep corpuscles; dose 0.5 c.c.

Sensitized sheep corpuscles—0.5 c.c. washed cells added to 1 c.c. inactivated immune serum and kept at 37 C. for 1 hour, then washed with salt solution; 2% suspension in salt solution.

Total volume in each tube brought 1 c.c. with salt solution. First and second incubations 1 hour each at 37 C.

Control tubes contained: (1) Antigen + corpuscles; (2) sensitizer + corpuscles; (3) complement + corpuscles; (4) corpuscles + salt solution; (5) complement + salt solution 37 C. 1 hour, then sensitized corpuscles 0.5; (6) sensitizer + complement + salt solution 37 C. 1 hour, then corpuscles suspension 0.5 c.c.

Exper. 1.—Seven determinant tubes contained each, antigen, sensitizer, complement, salt solution. Incubation for 1 hour at 37 C.; 0.5 c.c. corpuscles suspension added, again incubation for 1 hour.

Result: No hemolysis in any tube. Complete hemolysis in control Tubes 5 and 6.

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[3] Bordet: Studies in Immunity, Collected and translated by Gay, 1909.

Exper. 2.—Same as 1, substituting sensitized corpuscles for unsensitized. No hemolysis in any tube; complete hemolysis in Controls 5 and 6.

Exper. 3.—Substituting sensitized cholera vibrios, suspended in salt solution, for sensitized corpuscles. No Pfeiffer phenomenon in any determinant tube. Pfeiffer phenomenon complete in Controls 5 and 6.

Cholera Antigen

Heavy suspension in salt solution of cholera vibrios; suspension of sensitized vibrios; doses by titration 2 drops. Cholera antiserum (guinea-pig), inactivated, dose 1 drop, 2 units or 0.02 c.c. Cholera antigen, alcoholic solution, dose 1 drop, by weight same as in Expers. 1, 2 and 3. Other details same as in foregoing experiment, substituting cholera suspension for corpuscle suspension.

Exper. 4.—Six determinant tubes containing antigen, sensitizer, and complement salt solution. Incubation 1 hour at 37 C.; 2 drops vibrio suspension added; 2nd incubation for 1 hour. No agglutination or Pfeiffer phenomenon in determinant tubes. Agglutination and Pfeiffer phenomenon in Controls 5 and 6.

Exper. 5.—Same as 4, substituting sensitized cholera vibrios for unsensitized in 3 determinant tubes, and substituting sensitized sheep cells for vibrios in the last 3 tubes. No Pfeiffer phenomenon or hemolysis in any determinant tube; complete Pfeiffer phenomenon in Tube 5; complete hemolysis in Tube 6.

TYPHOID ANTIGENS

Similar experiments were carried out with the specific fats of the typhoid bacillus. The antigen was prepared as outlined previously (1000 sq. in. agar surface culture yielded 0.310 gm. of the sodium salts), using 5 strains of the organisms, and the serum came from animals immunized against typhoid, paratyphoid A and B, B. coli, and from persons sick with or convalescent from typhoid fever. These experiments showed that the typhoid fats were also specific, and that they gave fixation with paratyphoid B and with colon antiserums according to the subjoined details.

Exper. 6.—Antigen, alcoholic solution, 1 c.c. contained 0.002 gm.

Sensitizers, immune rabbit serum for typhoid bacilli, paratyphoid A and B, B. coli and human antityphoid serum, all inactivated.

Complement, sensitized vibrio and corpuscle suspensions, and other details the same as in the preceding experiments.

Rabbit: Antityphoid serum + +
Antiparatyphoid A serum —
Paratyphoid B serum ++
Anticolon serum, +

Human serum: Typhoid convalescent + +; agglutination +
Typhoid convalescent +; agglutination +
Typhoid convalescent +; agglutination +
Typhoid fever, 3rd week ++; agglutination +
Typhoid fever, 3rd week ++; agglutination +
Typhoid fever, 2nd week ++; agglutination +
Typhoid fever, 2nd week ++; agglutination +

Human serum: (continued)
Normal —
Normal —
Syphilitic —; Wassermann ++
Syphilitic —; Wassermann ++
Gonorrheic —; complement fixation ++
Gonorrheic —; complement fixation ++
Scarlatinal —
Scarlatinal —

Cross experiments wherein the specificity of the fat complexes was shown, and serving as negative controls in the preceding experiments, consisted in substituting the various antigens for the specific one in mixtures of the different immune serums and complement. These showed that complement is not bound save with the appropriate specific antigen and sensitizer, but that with antigens derived from germs of one species fixations occur with serums for related species. Indeed the range of the fats of the cells thus far studied has been shown not to be so great but that occasional fixation is given with an unrelated serum, by reason of the overlapping.

ANTIGENS OF BACILLUS TUBERCULOSIS

With regard to tuberculosis, experiments with the specific fatty antigen have shown an exception. At first sight it might appear that the failure of this antigen to bind complement in the presence of serum of patients suffering with tuberculosis constituted an exception fatal to the idea of the specificity of fats, especially in view of the results of serum tests in the hands of Craig and others[4] who have used antigens consisting of alcohol treated bacilli, aqueous suspensions and autolysates. On further consideration, however, it has become apparent that the exception tends rather to prove the rule, since I have been unable to show the presence of antibody in any serum from tuberculous patients. Moreover it has not been possible to obtain results at all comparable to those of the authors mentioned, using as antigens the various suspensions and preparations of tubercle bacilli made as nearly as possible according to their methods. As is shown in the accompanying table of representative tests we obtained results equally as good with suspensions of other organisms, and are forced to conclude that tests for antibody in the serums of tuberculous patients by the method of complement fixation with any antigen are unavailable and unreliable because of its frequent absence. This contention is supported by many facts in the literature.[3]

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[4] Craig: Jour. Am. Med. Assn., 1917, 68, p. 723. Corper: Jour. Infect. Dis. 1916, 19, p. 315. Corper and Sweeney: Jour. Am. Med. Assn., 1917, 68, p. 1598. Miller and Zinnser: Proc. Soc. Exper. Biol. and Med., 1916, 13, p. 134.

The following antigens have been employed and found to be of no value.

Alcoholic extracts of moist tubercle bacill at 25 C.
Alcoholic extracts of moist tubercle bacilli at 78 C.
Concentrated broth and 50% alcohol.
Alcohol and ether extracts of dry tubercle bacilli.
Tubercle poison (Vaughan), alcoholic solution.
Tubercle residue (Vaughan), aqueous suspension.
Tubercle extract, ether (Vaughan), alcohol soluble portion.
Tubercle extract, ether (Vaughan), alcohol-ether soluble portion.
Tubercle wax, melting point 45 C., alcohol-ether solution.
Tubercle alcohol, from saponified wax; hot alcohol solution.
Tubercle alcohol acetic ester alcohol solution.
Total fatty acids, alcohol solution.
Total sodium salts of fatty acids, alcohol solution.
Aqueous suspensions of tubercle bacilli.
Aqueous autolysates (salt solution).

CONTROLS

Syphilitic antigen.
B. Leprae (Duval), aqueous suspensions.
B. Typhosus, aqueous suspensions.
B. Anthracis, aqueous suspensions.
Bacillus X, from scarlatina, aqueous suspension.

The aqueous antigens were titrated so as to obtain a dose lying in the very narrow zone between the anticomplementary point and that giving no fixations at all. The alcoholic and ethereal extracts appeared to be anticomplementary in all doses. The addition of cholesterol increased this action, while the addition of the tubercle alcohol diminished it. By itself the alcohol possessed neither hemolytic, anticomplementary, or antigenic value in ordinary doses. The specific tubercle fats were not of themselves anticomplementary but were made so by the faintest traces of cholesterol. This was contrary to experience with the other antigens of Expers. 1-7, in which the addition of cholesterol gave sharper and more clearly cut inhibitions than without.

Examples of the results are given in Tables 1-4, in which ++ denote complete inhibition of hemolysis, + partial inhibition, and ± doubtful inhibition.

These results have been selected from many hundred tests as representing the best that is to be said for these antigens. The reactions were never sharp and clear cut. In a majority of instances it

TABLE 1

TESTS WITH TUBERCLE ANTIGENS *

Serums		Autolysate of Tubercle Bacilli	Sodium Salts of Fatty Acids from Tubercle Bacilli	Fatty Acids of Tubercle Bacilli
Active tuberculosis	1	±	—	—
	2	—	—	±
Chronic tuberculosis	1	+	+	++
	2	++	++	+
Syphilis		±	—	++
Normal	1	++	—	++
	2	—	—	—

* In this and the accompanying tables, ++ denotes complete inhibition of hemolysis, + partial inhibition, ± doubtful inhibition, and — absence of inhibition.

TABLE 2

TESTS WITH TUBERCLE ANTIGEN

Serums		Aqueous Suspensions of Tubercle Bacilli	Sodium Salts of Fatty Acids from Tubercle Bacilli	Fatty Acids of Tubercle Bacilli
Active tuberculosis	1	+	++	—
	2	±	++	—
	3	+	±	—
	4	++	±	+
	5	—	++	±
	6	±	—	±
Chronic tuberculosis	1	+	+	++
	2	+	±	±
	3	+	+	++
	4	+	++	++
	5	—	++	+
	6	++	++	+
Syphilis	1	—	—	—
	2	±	+	++
	3	—	±	—
Normal	1	++	±	++
	2	—	—	—
	3	—	++	++
	4	—	+	—
	5	++	+	+
	6	+	—	—

TABLE 3

TESTS WITH TUBERCLE ANTIGENS AND SUSPENSION OF BACILLUS X

Serums		Aqueous Suspension of Bacillus X from Scarlatina	Aqueous Suspension of Tubercle Bacilli	Autolysate of Tubercle Bacilli	Aqueous Suspension of Tubercle Bacilli	Autolysate of Tubercle Bacilli
Active tuberculosis	1	+	–	±	+	–
	2	–	±	+	+	–
Chronic tuberculosis	1	+	–	+	+	–
	2	+	±	±	+	+
Syphilis		–	+	++	++	+
Normal	1	+	±	+	+	+
	2	+	–	–	±	+

TABLE 4

TESTS WITH TUBERCLE AND OTHER ANTIGENS

Serums		Aqueous Suspension of						Wassermann	Gonococcus	Typhoid Bacilli (Agglutination)
		Tubercle Bacilli	Bacillus Leprae	Tubercle Residue	Typhoid Bacilli	Bacillus X Scarlatina	Anthrax Bacilli			
Active tuberculosis	1	–	–	–	++	++	–			
	2	–	±	–	–	++	–			
	3	+	+	±	++	+	–			
	4	+	–	±	±	–	–			
	5	±	+	±	±	+	±			
	6	±	±	–	±	+	–			
Chronic tuberculosis	1	±	–	–	+	++	–			
	2	–	–	–	±	++	±			
	3	±	++	–	+	+	–			
	4	+	+	–	–	–	–			
	5	±	++	–	++	++	+			
	6	+	±	+	+	++	–			
Typhoid fever	1	±	–	+	++	+	–			+
	2	±	±	–	–	±				+
Controls	1	+	±	+	–	–	++	++	++	
	2	±	–	++	–	–	++	++	++	
	3	+	±	+	–	+	±	–	++	
	4	–	–	–	–	–	±	–	–	
	5	±	±	++	–	–	++	–	+	
	6	+	+	+	–	±	++	–	–	

was necessary to set a time limit to the second incubation in order to obtain readings, and when the incubation was allowed to proceed in a manner consistent with the requirements of the controls by far the greater number of the determinants showed complete hemolysis. It is distinctly bad practice to accept such readings since they represent delayed hemolysis and not fixation of complement. When a positive reaction occurs with the syphilitic, the gonorrheic, the typhoid or other antigens mentioned there is no question about it. The cells settle to the bottom of the tubes and no amount of prolonged incubation or shaking alters the readings.

DISCUSSION

The antigenic rôle of lipoids in the immune reactions of agglutination, precipitation and complement fixation has been noted by many writers [3,5] especially those dealing with the serum reaction for syphilis. The presence of nitrogenous and other substances in the so-called lipoids has, however, justly supported the criticism that the impossibility of excluding such substances left the antigenic value of the fats themselves much in doubt. To the pure specific fats this criticism is not applicable, especially since in one instance at least an artificial fatty antigen from pure materials has been substituted,[6] and unpublished work indicates that many of the fatty complexes of bacterial and other cells can be assembled artificially.

It is not claimed and it is not likely that such fatty compounds represent the true state of the cell fats as they exist in the cells nor is it asserted that the form in which they are used in the reaction of fixation is the best possible form. Be this as it may it is claimed nevertheless that the complexes are available for antigenic use, that they represent the specific fat aggregates of the cells, and that the state in which they are used, in addition to their chemical configuration, is essential to their successful operation. The sensitizer-fatty antigen aggregate which favors the adsorption of complement is more readily brought about when the fats are in a certain physical state. For instance, the same fats as neutral fats will yield no complement adsorption while as fatty acids or as the alkali salts of the fatty acids the adsorption readily occurs. That the special or specific configuration of the fats is the key to the process of fixation is shown by the

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[5] Bang and Forsmann: Beitr. z. chem. Phys. u. Path., 1906, 8, p. 238. Warden: Jour. Am. Med. Assn., 1917, 68, p. 432.

[6] Warden: Jour. Am. Med. Assn., 1917, 68, p. 932.

fact that the slightest deviation from this configuration impairs the specificity of the test.

The rôle of these complexes of pure cell fats which are regarded as specific for each cell will be made clearer by holding a few well established facts in mind. We must regard the immune reaction of the fixation complement, the formation of specific precipitates, and the cytolytic properties of immune serums as embracing essentially the same phenomenon, that of the lytic action of complement on the surface of the cell sensitized by the appropriate sensitizer following its adsorption on the sensitized cell (Bordet). In other words, the action is unquestionably one directed on cell surfaces, or aggregate surfaces, or stromata in the sense of Bordet. Many bacterial and other cells, particularly those retaining the Gram stain, are known to contain fats at their surfaces, and from what is known of the chemicophysics of cells in general it appears to be safe to assume that the fats in one form or another constitute a large bulk of the stroma and surfaces, although by no means or necessarily to the exclusion of other substances such as protein and cholesterol, or other sterols.

Since now the presence of the cells themselves has been shown to be unnecessary and their functions assumed by the fats specific for those cells in the immune reaction we must conclude that sensitized antibody forms with the fat a specific aggregate which adsorbs complement in a manner wholly analogous to the process as worked out by Gay[3] for cell and other specific precipitates.

The application of these principles to the study of typhoid serum reactions will continue and form the subject for further communication. At the same time the idea is being extended to include other organisms, particularly the various strains of the pneumococcus.

SUMMARY

The value of antigens in serum tests for the presence of antibody in gonorrhea, syphilis, typhoid fever, and cholera appears to depend on fatty complexes of definite chemical arrangement or configuration which represent the fatty content of the several micro-organisms causing those diseases.

The reactions of the test are surface reactions and depend on the physical state of the antigen.

The serum test as applied to tuberculosis is fallacious and unreliable probably because of the absence of sensitizers in the serums.